

Summary of the doctoral dissertation 'Antibacterial and antifungal activity of extracts obtained from selected plant species'

The current state of civilization diseases, which include both mycoses and tuberculosis, is the result of the growing resistance of microorganisms to the available antibiotics, and their significant toxicity. Therefore, plants can successfully become a source of new therapeutics.

The aim of this study was to detect and analyze the antibacterial and antifungal activity of extracts: from sub-inflorescence stems of *Pelargonium zonale* and from *Sida hermaphrodita* seeds, which were selected from several extracts by prior determination of their lysozyme activity.

The extract from sub-inflorescence stems of *P. zonale* was effective and had a statistically significant impact on *M. smegmatis* cells at protein concentrations of 50-150 µg/ml, and on wild-type *C. albicans* cells at concentrations of 37.5-50 µg/ml. The effect of the extract on mycobacterial and yeast cells was analyzed using light, fluorescence, and electron microscopy. The action of the extract from sub-inflorescence stems led to a decrease in the survival rate of mycobacteria and to disintegration of bacterial cell walls. In the case of *C. albicans* cells a significant decrease in metabolic activity and, consequently, apoptosis and necrosis of some cells was observed after incubation with the extract. In addition, the effect of the extract of pelargonium stems on yeast cells include deformation of the cell wall, changes in the shape and size of the cells, formation of pseudohyphae and a unipolar pattern of cell budding, identified as a division disorders.

The *S. hermaphrodita* seed extract had a statistically significant effect on *M. smegmatis* cells at concentrations of 50-150 µg/ml, by reducing the viability of the bacteria and inducing changes in the structure of the mycobacterial cell wall. The analysis of the antifungal activity of the Virginia mallow seed extract consisted in examination of the effect of the crude seed extract, the extract after dialysis and extract fractions containing compounds with different molecular weights (below 30 kDa, in the range of 50-100 kDa, above 100 kDa) on *Candida* cells. It was found that both the crude *S. hermaphrodita* seed extract and the extract after dialysis significantly reduced the metabolic activity of wild-type *C. albicans* cells at concentrations of 12.5 - 200 µg/ml. Furthermore, the analysis of metabolic activity showed that the cells of the reference strain *C. albicans* ATCC 10231 and *C. krusei* ATCC 6258 were less sensitive to the action of the *S. hermaphrodita* seed extract.

In the case of the seed extract fraction, the greatest decrease in the metabolic activity of the yeast cells was recorded after the use of the fraction containing compounds with molecular weight exceeding 100 kDa (at concentrations of 25-100 µg/ml). The analyzes performed using light, fluorescence, transmission, and atomic force microscopy as well as infrared spectroscopy showed that the seed extract affected the cell wall of the wild-type *C. albicans* cells. This was confirmed by the structural changes in the cell wall, an increase in the average thickness of the cell wall compared to the control cells, and numerous deformations of the surface of the yeast wall observed in the microscopic image.

The analysis of the cytotoxicity against human skin fibroblasts showed that the extract of the *S. hermaphrodita* seeds, unlike the extract of the sub-inflorescence *P. zonale* stems, did not exhibit cytotoxic activity. Therefore, the next stage of the research was related to determination of the biochemical and proteomic characteristics of the seed extract. Using spectroscopic, spectrometric, and electrophoretic techniques, the protein-carbohydrate character of the Virginia mallow seeds extract was shown. Proteomic analysis carried out using MALDI, and ESI LC-MS/MS revealed the presence of proteins and peptides with structural, storage and enzymatic nature, in the extract, which are typical for seeds. Proteins and peptides with antimicrobial activity, identified as vicillins and lipid transporting proteins, were also determined in the protein profile of the extract.

Keywords: *Candida albicans*, *Mycobacterium smegmatis*, Virginia mallow, pelargonium, plant extracts, plant antimicrobial peptides

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